



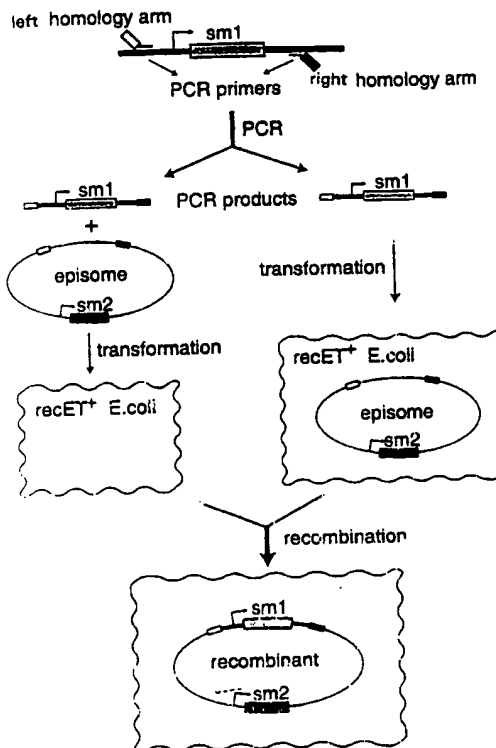
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|
| (51) International Patent Classification ⁶ : C12N 15/10, 15/90 | | A3 | (11) International Publication Number: WO 99/29837 |
| | | | (43) International Publication Date: 17 June 1999 (17.06.99) |
| (21) International Application Number: PCT/EP98/07945 | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). | |
| (22) International Filing Date: 7 December 1998 (07.12.98) | | | |
| (30) Priority Data: 97121462.2 5 December 1997 (05.12.97) EP 98118756.0 5 October 1998 (05.10.98) EP | | | |
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(54) Title: NOVEL DNA CLONING METHOD RELYING ON THE E. COLI RECE/RECT RECOMBINATION SYSTEM

(57) Abstract

The invention refers to a novel method for cloning DNA molecules using a homologous recombination mechanism between at least two DNA molecules comprising: a) providing a host cell capable of performing homologous recombination, b) contacting in said host cell a first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred. In particular, it relies on the use of the E. coli RecE and RecT proteins, the bacteriophage Red-alpha and Red-beta proteins, or the phage P22 recombination system. The beneficial effects of concomitant expression of the RecBC inhibitor genes (e.g. Red-Gamma) is also exemplified.



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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/07945

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/10 C12N15/90

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| X | OLINER J.D. ET AL.: "In vivo Cloning of PCR Products in E. coli" NUCLEIC ACIDS RESEARCH, vol. 21, no. 22, 1993, pages 5192-5197, XP002064297 cited in the application | 1-6, 8-13, 19, 20, 22-27, 34, 35, 37-42, 46-48, 50 18, 28 |
| Y | See page 5192, column 2, line 8 - line 31, and discussion section | |
| Y | NUSSBAUM A ET AL: "Restriction-stimulated homologous recombination of plasmids by the RecE pathway of Escherichia coli" GENETICS, vol. 130, no. 1, January 1992, pages 37-49, XP002103370 see figures 1,7 | 18, 28 |

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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Date of the actual completion of the international search

21 May 1999

Date of mailing of the international search report

04/06/1999

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INTERNATIONAL SEARCH REPORT

International Application No
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| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| P,X | ZHANG Y ET AL: "A new logic for DNA engineering using recombination in Escherichia coli" NATURE GENETICS, vol. 20, no. 2, October 1998, pages 123-128, XP002103371 see the whole document --- | 1-50 |
| A | KOLODNER R ET AL: "Homologous pairing proteins encoded by the Escherichia coli recE and recT genes" MOLECULAR MICROBIOLOGY, vol. 11, no. 1, 1994, pages 23-30, XP002064301 cited in the application --- | |
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